The Anti-inflammatory Effects of Tocotrienol in Macrophages

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INFLAMMATION

Inflammation is one of the immune response systems and plays an important role in damaged tissues and wounds which include other infections occurring at the site. Without the inflammation response, the wound would not be able to heal. Inflammation occurs as a result of the body's attempt in self-protection, in order to remove harmful stimuli and to initiate the healing process. This is identified as a defence mechanism in our body in recruiting the immune cells to the site of the inflammation (Nordqvist, 2017). To start the healing process, our immune system is required to differentiate between normal cells and damaged cells, irritation, pain and also pathogens. The biological responses will continuously try to remove any harmful substance or irritation that affects a part of our body. When there is a harmful substance or inflammation present in our body, a biological response will start spontaneously and try to recruit our immune cells to the site of injury, sometimes causing pain and discomfort. This is an indication that our body is trying to heal itself (Nordqvist, 2017). However, if inflammation persists longer than necessary, it can cause more harm than benefit to the body (Nordqvist, 2017).

The initial stage of inflammation is usually called an irritation, and is followed by the inflammation stage of the cells. Discharge of pus will then follow, and progress to the granulation stage before new tissues are formed at the site of injury. During the inflammation process, macrophages proliferate, differentiate or become activated in order to end the proliferation stimuli. Without inflammation, damaged cells such as those in infections and wounds will never start to heal. Inflammation occurs when the body suffers aggression either by microbes or trauma by physical agents such as radiation, heat, etc. The inflammation can lead to a higher degree of cellular and tissue damage if there is an imbalance between the inflammatory signals that initiate and maintain the inflammation and a further step to shut down the process.

Macrophages are considered as a main part of the mononuclear phagocyte system, which consists of bone marrow cells, monocytes and some macrophage tissues. The macrophages actually come from our blood cells, i.e. monocytes, which migrate into various tissues (Fujiwara and Kobayashi, 2005). Under normal conditions, only a few cells differentiate in response to stimuli, converting mature cells or tissue-specific cells such as dendritic cells, kupffer cells (specialised macrophages located in the liver) and microglia, while others are removed by apoptosis.

INFLAMMATION AND MACROPHAGES

Macrophages have three major functions during inflammation, which are antigen presentation, phagocytosis and immunomodulation through the production of various cytokines and growth factors (Fujiwara and Kobayashi, 2005). Macrophages play an important role in the initiation, maintenance and resolution of inflammation. Their main contribution is to innate immune response, which is generally non-specific and develops after infection or vaccination. In the initial stage of inflammation, macrophages will destroy the remaining microbes that escape the neutrophils, and will remove apoptotic bodies of dead neutrophils and present antigens to T lymphocytes, thus initiating the mechanisms of acquired immunity by producing antibodies, cytokines and memory cells (Celada, 2008). They are activated and deactivated in the inflammatory process. Activation signals include cytokines

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(Interferon gamma, granulocyte-monocyte colony stimulating factor and tumour necrosis factor alpha), bacterial lipopolysaccharide, extracellular matrix proteins, and other chemical mediators. In certain circumstances, such as in chronic inflammation, macrophages have a harmful effect rather than fixing the problem, and cause lesions instead.

**ACUTE AND CHRONIC INFLAMMATION**

Acute inflammation is considered a short-term process which appears within minutes and can extend to several hours. This is induced by several types of tissue damage caused by trauma, toxic compounds, or microbial attack. The acute inflammation process is facilitated by immune cells, important cell-signalling proteins called cytokines, and other small molecules. Anaphylatoxins, small immune-mediating molecules released at the site of inflammation, stimulate mast cells to release histamine, serotonin and prostaglandins, which cause blood vessels to expand (vasodilation) and become more penetrable. This response allows immune cells such as neutrophils to migrate into the affected tissue through the capillary wall (diapedesis) and respond to the offending agent (Figure 1). Neutrophils are the major component of pus, and additional clinical signs of acute inflammation include swelling, redness, pain and heat at the site of the injury.

By contrast, chronic inflammation refers to long-term inflammation and can extend to several days, months or years. This inflammatory response can be seen in certain viral infections and hypersensitivity reactions, especially if the cause of inflammation is persistent (Table 1). In chronic inflammation, macrophages and T lymphocytes are the main important components of the primary immune cells which produce cytokines and enzymes that trigger more harm to the damaged cells. In chronic inflammation, resolution of the acute infection gives way to ongoing tissue damage and destruction, manifesting as tissue fibrosis.

These molecules which are found in acute and chronic inflammation have been investigated for pharmacologic interventions. Chronic inflammation can lead to cancers and other diseases such as heart disease and stroke, as well as autoimmune disorders including rheumatoid arthritis and lupus.

A macrophage is the primary cell of the immune system and is an actively involved immune cell that acts by elongating its false

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**TABLE 1. CHARACTERISTICS OF ACUTE vs. CHRONIC INFLAMMATION**

<table>
<thead>
<tr>
<th>Acute Inflammation</th>
<th>Chronic Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cells</strong></td>
<td></td>
</tr>
<tr>
<td>Infection: Neutrophils.</td>
<td>Maccrophages, lymphocytes</td>
</tr>
<tr>
<td>Allergy: Eosinophils, mast cells</td>
<td></td>
</tr>
<tr>
<td><strong>Chemical Mediators</strong></td>
<td></td>
</tr>
<tr>
<td>Complement, kinins, prostaglandins, leukotrienes, cytokines (Interleukin 1, Interleukin 6) from various immune cells, interferon-gamma from T cells</td>
<td>Cytokines from macrophages and T lymphocytes</td>
</tr>
<tr>
<td><strong>Lesion</strong></td>
<td></td>
</tr>
<tr>
<td>Rash, pus, abscess</td>
<td>Rash, fibrosis, granuloma</td>
</tr>
<tr>
<td><strong>Clinical Examples</strong></td>
<td></td>
</tr>
<tr>
<td>Abscesses (brain; skin), allergic reaction (anaphylaxis)</td>
<td>Autoimmune conditions (lupus; rheumatoid arthritis), cystic fibrosis</td>
</tr>
</tbody>
</table>

Modified and adapted from Khan Academy. Retrieved from https://www.khanacademy.org

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**Figure 1. Neutrophil diapedesis. The role of neutrophils and monocytes in innate immunity [adapted from Kantari et al.(2008)].**
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feet to encourage phagocytosis of the agents to permit clearance and maintain an ideal environment for optimum body function (Muniandy et al., 2018; Hou et al., 2015). The major aim of inflammation is to get rid of the detrimental agents from our body. When a foreign material is present, it causes a specific binding protein on the body’s cell surface to fix and form complexes known as pathogen-associated molecular patterns.

In responding to the existence of cellular stimuli, the inflammatory signalling as well as the transcribing of pro-inflammatory genes are activated, therefore generating pro-inflammatory mediators and cytokines, including nitric oxide (NO), cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), interleukin 6 (IL-6), interleukin 1β (IL-1β), tumour necrosis factor alpha (TNFα), and prostaglandin (PGE2) (Fard et al., 2015).

The activated inflammatory pathway results in an increase in pro-inflammatory products that are involved in the process of pathological pain, which is vital for a better immune response. Their accumulation may act as a positive feedback mechanism that triggers the release of more of the pro-inflammatory entities, because they can act as the inflammatory stimuli to activate a signalling pathway (Martiney et al., 1998). Over time, prolonged inflammation may cause damage to the tissues around the affected region.

TOCOTRIENOLS

Tocotrienols are considered as one of the vitamin E family. The two types of vitamin E isoforms are called tocopherols and tocotrienols. Vitamin E is a substance necessary for proper body and brain function. Tocotrienols can be found in the oils of rice bran, palm fruit, barley and wheat germ. These substances are also available in supplement form as capsules or pills. Tocopherols, however, are found generally in vegetable oils such as olive, sunflower and safflower oils, whole grains, and green leafy vegetables.

Researchers believe that tocotrienols have many health benefits, and are considered to be more powerful antioxidants compared to tocopherols. These benefits include increased brain health and functionality, anticancer activity, and cholesterol-lowering properties. The positive effects of tocotrienols as being hypocholesterolemic, having antioxidant and anti-inflammatory effects (Qureshi et al., 2010; Yam et al., 2009) and as anticancer agents have been confirmed in animal systems and various cell lines by many investigators (Qureshi et al., 1993; Nesaretnam et al., 1998; Parker et al., 1993).

It is suggested from scientific studies that tocotrienols have several additional health benefits which include:

• **Improving fracture healing.** A study on post-menopausal rats with osteoporosis has shown that tocotrienols helped to strengthen and heal bone fractures faster than other vitamin-E based supplements.

• **Neuro-protective effects.** A study showed that tocotrienols quickly and easily reached the human brain, and may improve brain function and health.

• **Anti-cancer effects and increasing overall health.** A study proposed that tocotrienols have a positive effect on human health, and have anticancer properties.

• **Reducing the risk of cardiovascular disease and lowering cholesterol levels.** Tocotrienols assist in reducing the accumulation of plaque in arteries and lowering cholesterol levels.

Furthermore, the efficacy of tocotrienols versus tocopherols as antioxidants has been established, and δ-tocotrienol was shown to be the most potent amongst the known tocotrienols (Qureshi et al., 2000; Serbinova et al., 1991). Tocotrienols also exhibit non-antioxidant properties in various in vitro and in vivo models which interact with the mevalonate pathway that leads to the lowering of cholesterol levels, the prevention of cell adhesion to endothelial cells, the suppression of tumour cell growth (Hafid, 2014; 2013), and glutamate-induced neurotoxicity (Khanna et al., 2007; Theriault et al., 2002).

Our investigation was carried out to evaluate the mechanisms by which tocotrienols inhibit inflammation in macrophages, especially in RAW 264.7 macrophages. These experiments were designed to test our hypothesis that tocotrienol treatment modulates the pro-inflammatory activity and cytokines such as COXII, IL-6 and TNF-alpha, and blocks LPS-induced signalling pathways that contribute to the inflammatory process (Qureshi et al., 2010).
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According to our study, it was demonstrated that treatment of RAW 264.7 macrophages with 10 ug ml\(^{-1}\) of TRF down-regulated the expression of the COX-II genes compared to the control sample as can be seen from Figure 2(a). The intensity of the band decreases with increasing TRF concentration. We also measured interleukin 6, IL-6 in the cell supernatant. Treatment cells with 10 ug ml\(^{-1}\) TRF decreased the IL-6 level in Raw 264.7 macrophages after incubating for 24 hours [Figure 2(b)]. For TNF-alpha gene expression, it can be observed that the band intensity in 10 ug ml\(^{-1}\) of TRF decreased compared to the control with stimulation, and there was a further decrease in the band intensity at the higher TRF concentration of 20 ug ml\(^{-1}\). The expression of TNF-alpha was almost comparable with that of the commercial drug indomethacin [Figure 2(c)]. The results suggest that TRF possesses anti-inflammatory effects that target the COX pathway, TNF-alpha gene expression and the production of cytokines such as IL-6 are able to protect the macrophages from LPS-induced cytotoxicity.

CONCLUSION

The results of our current study show the capacity of tocotrienols to modulate inflammation. The anti-inflammatory properties of tocotrienols can be further evaluated in future studies as well as the effects of single isomers of tocotrienols on macrophages for better understanding and greater benefits in human health.

REFERENCES


Hafid, S R A; Radhakrishnan, A K and Nesaretnam, K (2010). Tocotrienols are good adjuvants for developing cancer vaccines. BMC Cancer, 10: 5.


Hou, X L; Tong, Q; Wang, W Q et al. (2015). Suppression of inflammatory responses by dihydromyricetin, a flavonoid from Ampelopsis grossedentata, via inhibiting the activation of NF-κB
and MAPK signaling pathways. J. Natural Products, 78(7): 1689-1696. DOI: 10.1021/acs.jnatprod.5b00275.


